IN VITRO STUDIES ON VITILIGO*

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Vitiligo is an acquired condition which is associated with the loss of pigment without other apparent histological changes in the skin. In general, it is believed that the melanocytes actually are present in the involved skin but are in an inhibited state of functional activity. Our present study reports on the cytological findings of various pigment cells in the normally pigmented and depigmented skins of 18 cases of vitiligo. We also made cytological observations on the melanocytes of the repigmented areas in vitiligo patients who had either repigmented spontaneously or following treatment.

MATERIALS AND METHODS

Split thickness skin specimens were obtained from areas which had been infiltrated with Xylocaine®. The skins, thus obtained, contained the whole epidermis and a very small amount of dermis. A definite attempt was made to take our normal control specimens from an area of skin which was similar in location to that which was vitiliginous. This was done in order to obtain a better appreciation of any increase or decrease in number of melanocytes present. The difference in number of melanocytes in different locations in the same individual is well known (1, 2).

Specimens were again taken anywhere from a few months to 2 years following treatment with systemic methoxsalen in conjunction with ultraviolet light. If possible, these specimens included: 1) repigmented sites, 2) vitiligo areas which failed to repigment, 3) mottled areas with isolated spots of perifollicular repigmentation, and 4) the border between pigmented and nonpigmented areas.

The specimens were then divided into two parts and subjected to the following studies:

I. Splitting with sodium bromide

Both normal and vitiliginous skins were split with 2N sodium bromide solution, following the procedure described by Staricco and Pinkus (2).

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By this technic the epidermis was easily separated from the corium. The sheet of epidermis was then subjected to the following reagents:

1) Dopa reaction (3).

2) Silver reduction technic for the demonstration of argentaffine granules (4). A slight modification of Masson's ammoniacal silver nitrate technic was employed; this appeared to give more consistent results in our hands.

3) Supra-vital staining with methylene blue (5).

II. Tissue culture studies

The skin biopsy specimens were cut into pieces approximately 2 x 2 mm. square and explanted with the roller tube tissue culture method (6). After incubation at 37° C. for three to four weeks, the cultures were studied with the same procedures employed in the split skins. In addition to these technics, the specimens were stained for detailed cytological examination with May-Gruenwald-Giemsa reagents (7).

RESULTS

Among a total of 18 cases of vitiligo studied, 15 were diagnosed as typical vitiligo, 2 cases as congenital vitiligo, and one case as pseudovitiligo.

I. Split skin examinations—The results were summarized in Table I

1) Typical vitiligo

On examination, there were no histochemically recognizable melanocytes observed in the vitiliginous skins of all 15 of our patients with typical vitiligo; normal melanocytes were present in the normally pigmented skins of these individuals (Fig. 1). In the vitiliginous areas, however, there were small, round, ovoid or slightly stellate cells which appeared more or less homogeneously gray or black in the dopa treated specimens (Fig. 2). Morphologically, these cells resembled the dopa-positive melanocytes seen in Caucasian or lightly pigmented skins. However, they differed from the latter in that they did not have a granular cytoplasm. These cells probably represent "inactive" melanocytes. It must be kept in mind that almost all cells take up some brownish black coloration when subjected to prolonged

TABLE I

Cytological findings in different cases of vitiligo studied

	Total Number of Cases	Typical Melanocytes	"Inactive" Melanocytes	Argentaffine Granules	Repigmentation
Typical Vitiligo.....	15	0/15	11/15	2/15	marked improvement in 2 cases with argentaffine granules
Pseudo Vitiligo.....	1	1/1		1/1	complete
Congenital Vitiligo.....	2	1/2	1/2	2/2	marked improvement in 1; repigmentation in the other*

* This case has not been followed long enough to be certain how complete the repigmentation is going to be.

incubation in the dopa reagent. This black coloration is due to a non-specific darkening effect of the chemical and is not a true dopa reaction. A dopa-positive melanocyte may appear to be almost solid black when it is strongly reactive, but if examined carefully, it is always somewhat granular in appearance. In the less intense reaction, the granularity of the cytoplasm is very apparent. We feel that cytoplasmic granularity is an important requisite for a true positive dopa reaction. In the vitiliginous areas in 11 out of the 15 skins examined there were a few of the homogeneously black stained small cells mentioned above. However, no typical dopa-positive melanocytes were observed (Figs. 3, 4, 5).

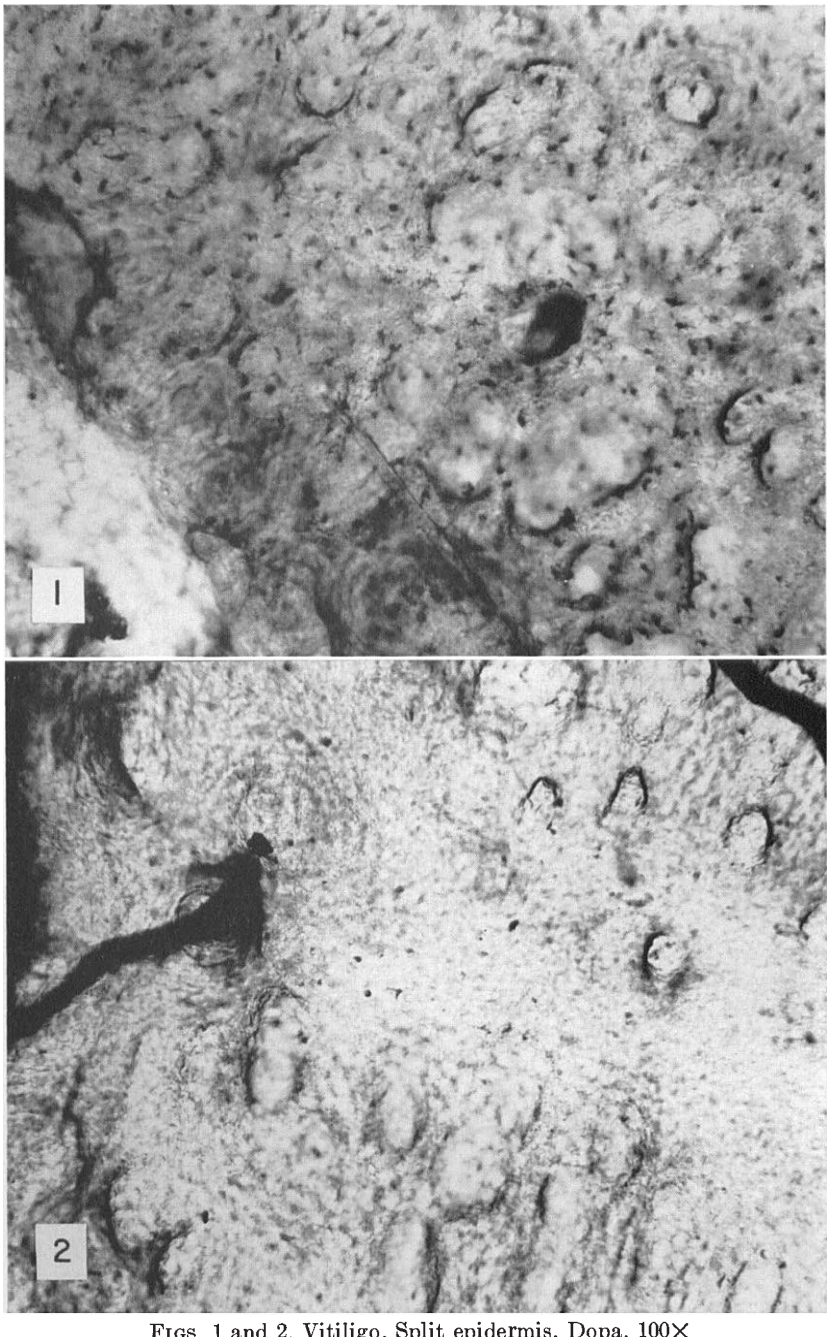
Supra-vital methylene blue staining of the vitiliginous skins revealed the presence of small numbers of isolated cells which were either round, ovoid, or stellate in shape and which appeared to take a little more blue staining than the adjacent polyhedral epithelial cells. However, it was impossible to always distinguish small stellate cells from isolated epithelial cells. We were not positive whether these cells represented the so-called "white" dendritic cells as described by Billingham (5) or whether they simply represented epithelial cells dissociated incidental to the chemical splitting of the preparation.

The rounded to slightly stellate cells which were found in the vitiliginous areas apparently did not fulfill all the criteria which we customarily use to define a melanocyte; therefore we do not feel justified in classifying them as normal melanocytes. If we can be reasonably sure that they are not dissociated epithelial cells, then we can tentatively consider them as "inactive" melanocytes. From this point on we will refer to these cells as such in this paper.

Silver treated preparations gave the following findings. With the exception of two cases which will be discussed later, no silver positive granules were present in any of the vitiliginous skins examined.

In 2 of our 15 patients with typical vitiligo, there were findings which deserved special attention. Prior to the institution of any treatment we found small islands of cells with intracytoplasmic argentaffine granules in the vitiliginous areas. The dopa reaction in the same areas was negative. It is indeed interesting that these 2 patients were found to clinically repigment far more readily and more extensively following methoxsalen and ultraviolet therapy than did any of our other patients. These 2 patients were unique in other respects; they also failed to lose any of their repigmentation when methoxsalen and ultraviolet light were discontinued for 6 months in one case and for 2 years in another. Both of these individuals were non-Negro dark-skinned people.

After treatment with methoxsalen and ultraviolet light, normal silver positive, dopa-positive, and methylene blue positive melanocytes were seen in both normally pigmented and repigmented areas. There was no discernible morphological difference between the melanocytes in the normally pigmented and the repigmented areas. These repigmented areas were of the same tone or shade as the patient's normal skin. The areas which failed to repigment after therapy gave negative reactions to silver, dopa, and methylene blue. The mottled areas of repigmentation and hyperpigmented borders of the vitiliginous patches revealed melanocytes which were positive with silver, dopa, and methylene

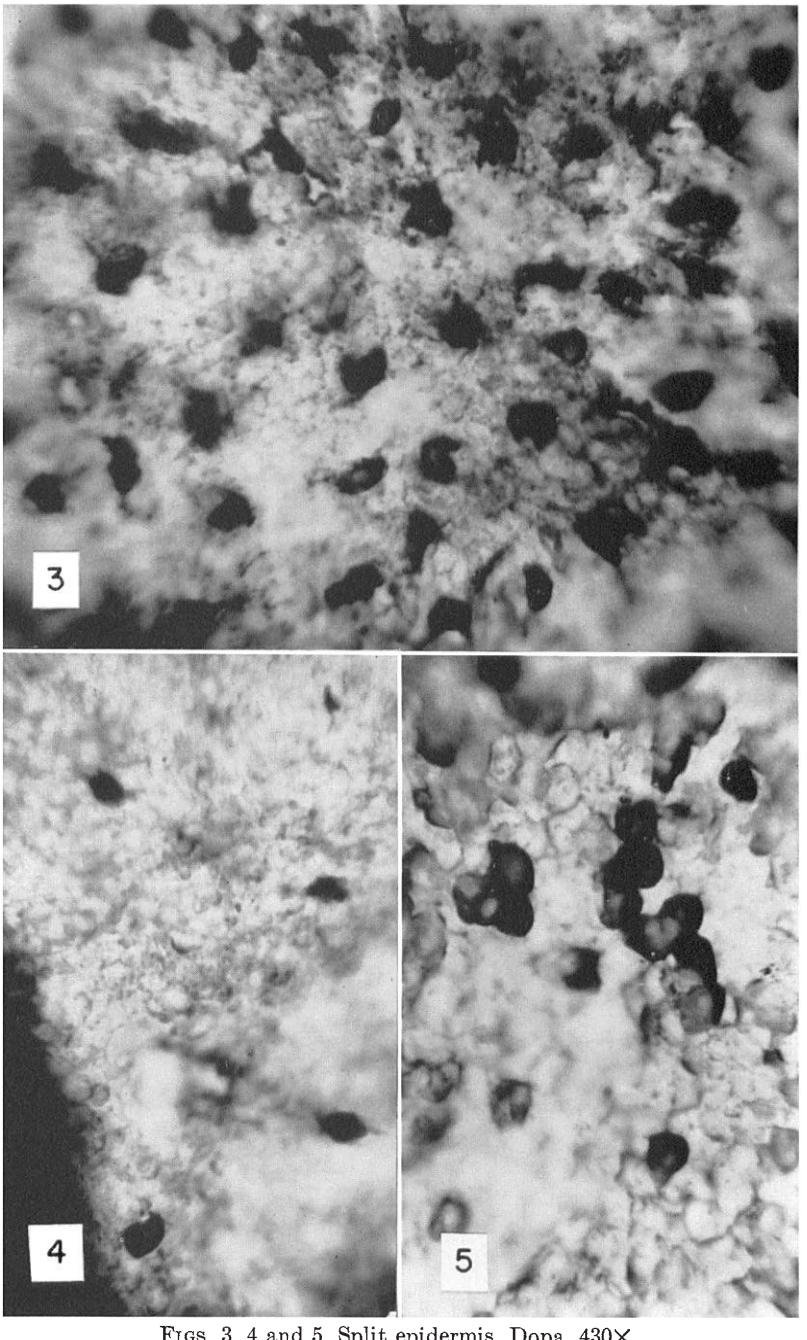


FIGS. 1 and 2. Vitiligo. Split epidermis, Dopa. 100×

FIG. 1. Normally pigmented skin in vitiligo patient. Note the evenly distributed dopa-positive melanocytes in the split epidermis.

FIG. 2. Depigmented skin in the same patient. Note the few scattered dark cells.

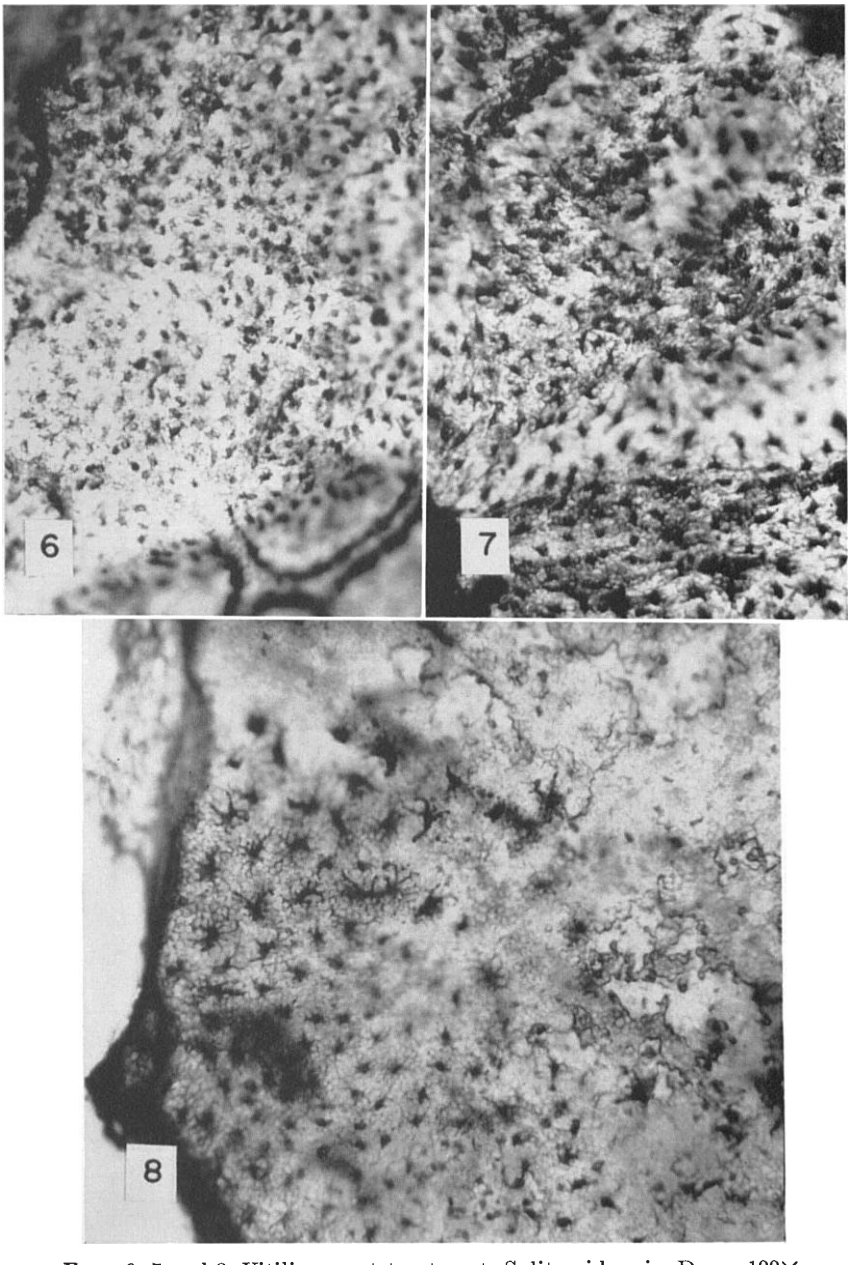
blue. These melanocytes were distinctive in that they were larger and more dendritic and more complex than normal melanocytes (Figs. 6–12). Clinically, the skin in these areas was more darkly pigmented than the normal skin. Extensive *in vitro* examinations were done in one of these 2 patients. The findings were summarized in Table II.



FIGS. 3, 4 and 5. Split epidermis, Dopa. 430×
FIG. 3. Normally pigmented skin in vitiligo patient.
FIG. 4. Depigmented skin in the same patient. Note the small dark "inactive" melanocytes.
FIG. 5. Depigmented skin in the same patient. The small aggregates of dark cells are keratinized epithelial cells which have stained non-specifically with the dopa reagent.

2) Pseudovitiligo

This patient was so designated because he did not fulfill all of the clinical and laboratory criteria for true vitiligo. His lesions consisted of hypo- rather than depigmented macules with poorly demarcated borders close to the midline of the back (Fig. 13). Histopathological examinations of a biopsy taken from one of these lesions



FIGS. 6, 7 and 8. Vitiligo, post-treatment. Split epidermis, Dopa. 100×

FIG. 6. Normally pigmented skin.

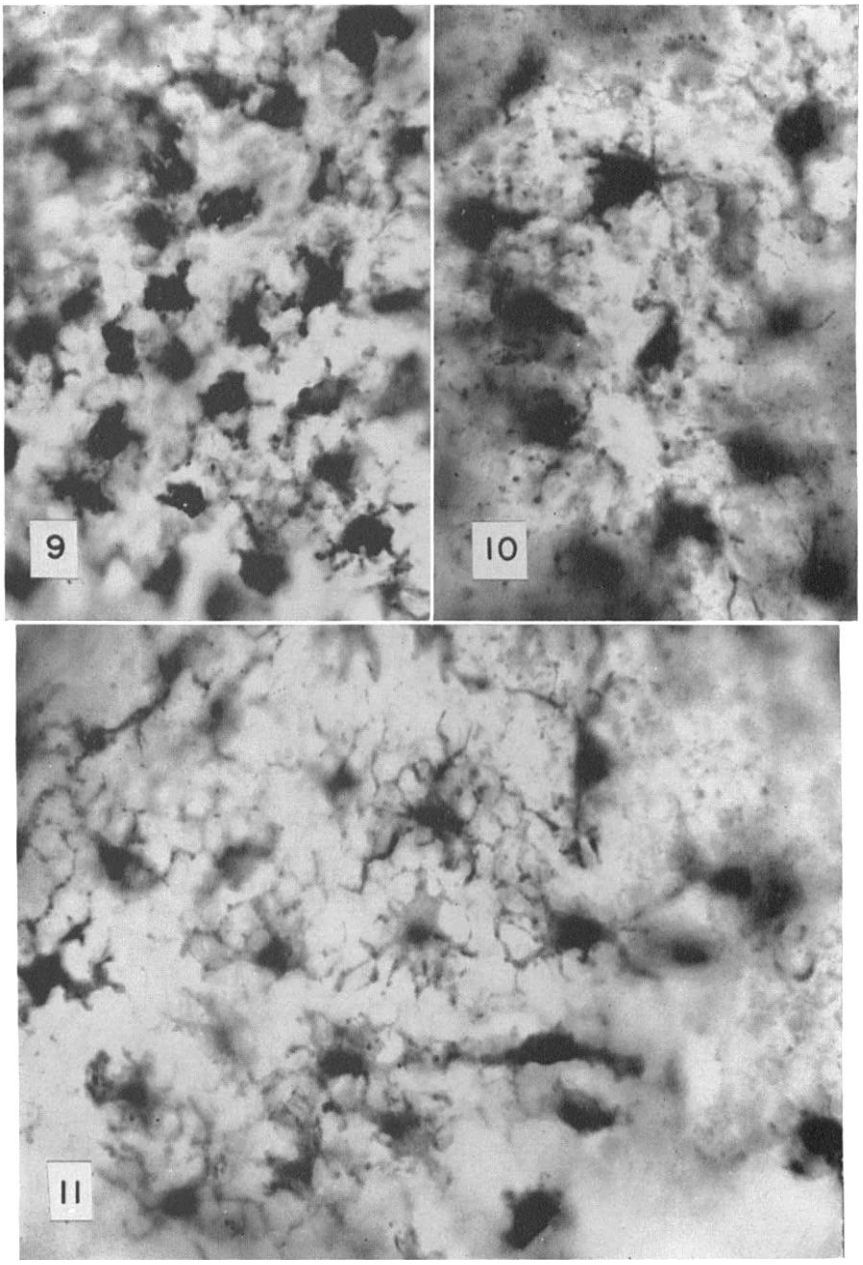
FIG. 7. Mottled repigmented skin. Note the cells are larger than the melanocytes in the normally pigmented skin.

FIG. 8. Vitiliginous skin with hyperpigmented borders. Note the large polydendritic dopa-positive melanocytes at one side and small "inactive" melanocytes on the other.

were normal. Skin scrapings were negative for *T. versicolor*. As mentioned above, the borders were poorly demarcated and not hyperpigmented as we so often see in vitiligo.

Dopa positive melanocytes and cells containing argentaffine granules were present in the split skin specimen from the hypopigmented macules. However, the dopa reactions and the silver reactions were slightly less uniform and less intense in the involved skin than in the normally pigmented skin of the patient (Figs. 14, 15).

It is interesting that this patient failed to



FIGS. 9, 10 AND 11. Vitiligo, post-treatment. Dopa. 430×
FIG. 9. Normally pigmented skin.
FIGS. 10 AND 11. Mottled repigmented skins. The melanocytes in this area are larger and more dendritic than those in the normally pigmented skin of the same patient.

repigment following local methoxsalen and intense artificial ultraviolet light exposures once weekly for 6 weeks. However, he repigmented completely within 2 weeks while sunbathing in Florida. This was accomplished without the use of psoralens in any form.

3) Congenital vitiligo

These cases were previously diagnosed as localized albinism. Both of these patients had depigmented patches present at birth. We will discuss these 2 cases separately.

The first was a 16 year old white girl with a

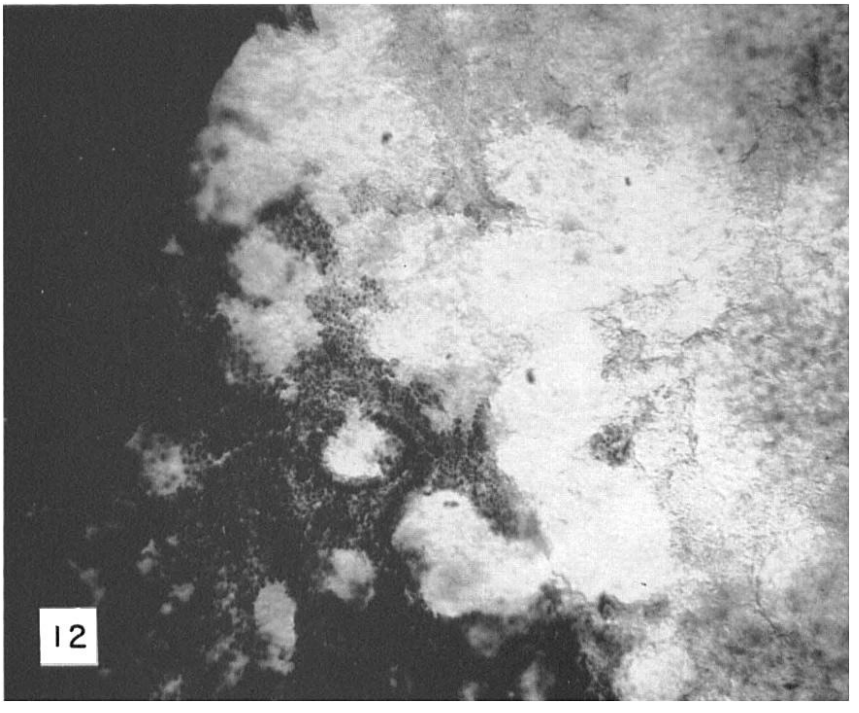


FIG. 12. Vitiligo, border area. Split epidermis, Masson's silver technic. 100×
Note sheets of silver positive epidermal cells in the hyperpigmented skin in contrast to the silver negative vitiliginous area. There are a few islands of weakly silver positive cells in the intermediate zone.

TABLE II

Results after oral methoxsalen and ultraviolet light therapy in one patient

	Normal Skin	Repigmented Areas	Depigmented Areas	Mottled Areas or Hyperpigmented Borders
Silver	positive	positive as control	negative	positive in areas
Dopa	positive	positive as control	negative	positive in areas large polydendritic cells
Methylene blue	positive	positive as control	negative	positive in areas large polydendritic cells

large depigmented patch on the left leg. When first seen in our clinic she had already begun to show some evidence of spontaneous repigmentation. Prior to the institution of methoxsalen therapy, dopa-positive melanocytes as well as cells containing silver positive granules were present in the skin of the depigmented areas and in areas which appeared to be repigmenting (these areas were darker than her normal skin). A biopsy specimen taken for histological examination from the darker areas failed to reveal the presence of nevus cells. The melanocytes in the depigmented and in the normal skin were similar in morphology and staining reactions. These melanocytes would be classified as normal melanocytes. As would be expected, the melanocytes in the hyperpigmented areas were larger, more complex and dendritic than those seen in her normal skin. These cells resembled those pigment cells activated by irradiation. We were not surprised when this patient repigmented almost completely with systemic methoxsalen and ultraviolet light therapy, since her pretreatment split skin specimen revealed the presence of large numbers of cells containing argentaffine granules as well as dopa-positive cells. The

FIG. 13. Pseudovitiligo

repigmented areas in this patient revealed the presence of both normal and the larger, more dendritic melanocytes.

The second case was a Negro girl, aged 15, who was born with a large area of depigmentation on the dorsum of the right hand; 4 months prior to visiting our clinic she developed new areas of depigmentation on her face and anterior chest. At the time of our examination there was no evidence of spontaneous repigmentation as evidenced by the lack of hyperpigmented borders or of islands of repigmentation within the patch. Examination of split skin prior to therapy revealed islands of silver positive cells as well as "inactive" melanocytes; there were no typical dopa-positive cells in these preparations (Figs. 16, 17). After the patient had had methoxsalen therapy for 3 months small islands of repigmentation were clinically evident inside of the patches of recent vitiligo with no definite evidence of repigmentation in her congenital patch.

At the time of this writing no split thickness specimens have been taken at the area of repigmentation.

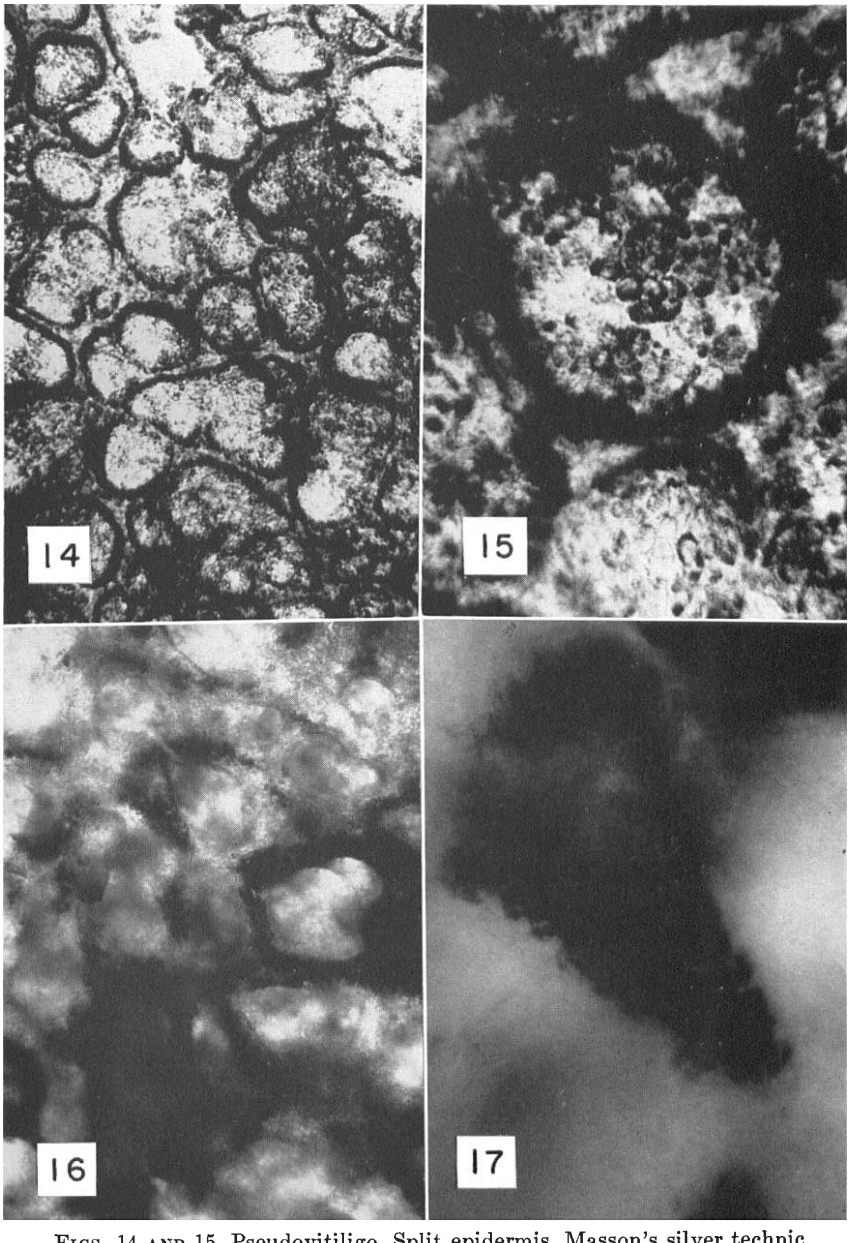
II. Vitiligo in tissue culture

When skin specimens from vitiliginous areas were cut for explantation, the depigmented skins were found to be more firm than normal and difficult to cut. This skin appeared to be more fibrous than the normal skin from the same area. In tissue culture the predominant outgrowth from vitiliginous skins appeared to be fibrocytic; only rarely did extensive epithelial sheet outgrowth occur. In contrast, normal skin outgrowth is more epithelial than fibrous. These findings appeared to indicate some mesodermal component in vitiligo.

The tissue culture specimens when treated in a manner similar to split skin specimens gave comparable results.

DISCUSSION

Repeatedly, it has been demonstrated that melanocytes are present in approximately equal numbers in the skins of all races. The melanocytes are identified by their morphological characteristics and staining reactions. At present there are only 2 ways of positively identifying a melanocyte: 1) the dopa reaction, and 2) a positive silver reaction consisting of intracytoplasmic argentaffine granules. The exact nature and functional significance of the questionable dopa-positive small cells which we have tentatively called "inactive" melanocytes in this paper remain obscure. These cells appear to be smaller and less stellate than normal melanocytes.



FIGS. 14 AND 15. Pseudovitiligo. Split epidermis, Masson's silver technic
FIG. 14. Hypopigmented skin. 100×. Note the relatively uniform distribution of the silver positive cells
FIG. 15. Same. 430×
FIGS. 16 AND 17. Congenital vitiligo. Split epidermis, Masson's silver technic. Islands of silver positive cells.
FIG. 16. Depigmented skin. 100×. Compare the distribution of the silver positive cells with that of pseudovitiligo.
FIG. 17. Same. 430×

and are found sparsely scattered in preparations of vitiliginous skin. In 2 of our cases of typical vitiligo we found small islands of cells containing argentaffine granules in their cytoplasm. The positive silver reaction in these cells tends to indicate some enzyme activity in the pigment cells. It should be noted that the arrangement of these cells containing argentaffine granules

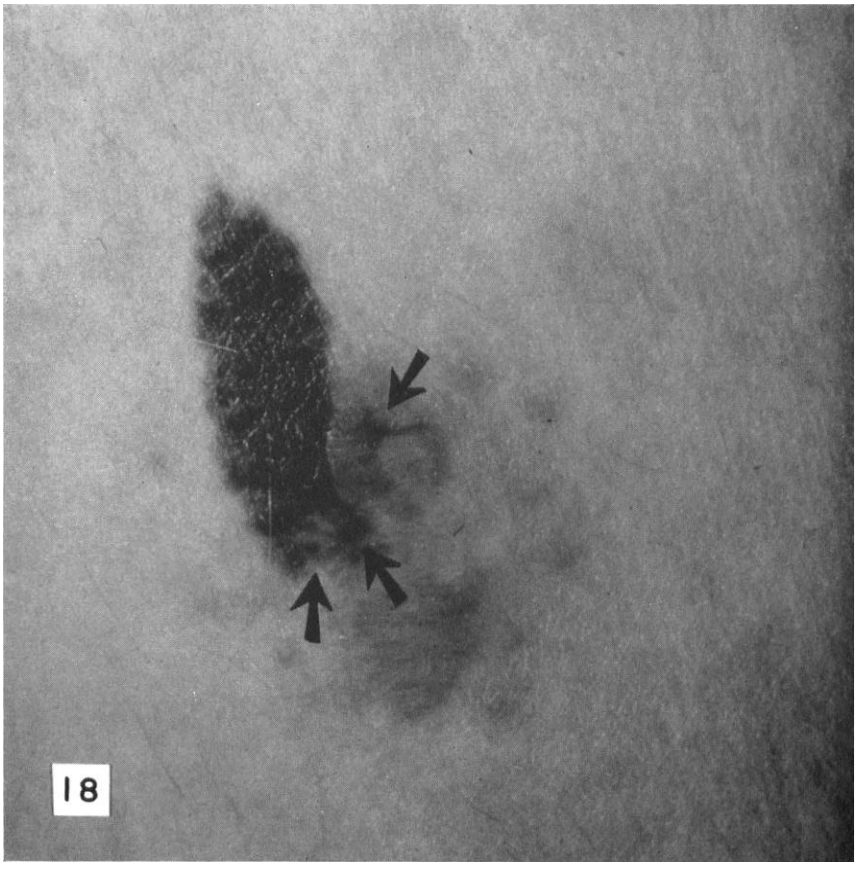


FIG. 18. Pigment spread into the scar of a partially excised compound nevus

differs markedly from the arrangement of the melanocytes in normal and from the "inactive" melanocytes in vitiliginous skin. These cells are arranged in small islands or groups in contrast to the single cell arrangement of the melanocytes shown in the dopa reaction. We do not believe these islands of cells are entirely made up of melanocytes; on the contrary we are of the opinion that the islands consist of both melanocytes and epithelial cells. It appears significant that these two cases of vitiligo were relatively good pigmenters under the influence of methoxsalen therapy.

With the exception of the 2 cases just mentioned, there was an absence of typical dopa-positive melanocytes as well as an absence of islands of cells containing argentaffine granules in all our cases of typical vitiligo. It is our impression that these were the vitiliginous patients whose repigmentation was classified as clinically unsatisfactory.

Jarrett and Szabo (8) classified vitiligo into 2 main types, i.e. absolute and relative. The absolute type was found to be completely lacking in dopa-positive melanocytes. We believe the so-called absolute vitilige of Jarrett and Szabo does contain "inactive" melanocytes. These authors classified the relative type of vitiligo into 2 subtypes. Subtype I had melanocytes which were slightly dopa-positive and which were present in normal numbers; subtype II had a reduced number of larger, more dendritic dopa-positive melanocytes. With respect to subtype I, we found only one case of vitiligo (not included in this study) which exhibited relatively normal numbers of "inactive" melanocytes with a questionable dopa reaction. All our cases included in this study showed reduced numbers of these cells. With respect to subtype II, it is our impression that the larger, more dendritic dopa-positive melanocytes are found only in the pigmenting borders and in sites

showing perifollicular repigmentation. These hyperpigmented borders and islets of repigmentation are found both in patients repigmenting spontaneously (usually in the summer) and in patients receiving therapy with methoxsalen and ultraviolet light. Most vitiligo patients will show this type of melanocyte in some areas of their skin. We do not believe that the presence of the hyperpigmented border and/or the presence of the larger, more dendritic dopa-positive melanocytes necessarily indicates a good prognosis for repigmentation.

In our studies, it appears that the presence of silver positive cells in a vitiliginous area is a better indicator for predicting repigmentation than is the presence of dopa-positive cells of any type, large or small. Our 2 cases of typical vitiligo which responded readily to therapy were those in which islands of cells with silver positive granules were observed. It is interesting that the one case of pseudovitiligo which contained both dopa-positive and silver positive cells repigmented completely on exposure to natural sunlight. These aggregates of silver positive cells were also present in the 2 cases of congenital vitiligo which repigmented markedly with treatment.

The fibrous nature of the vitiliginous skin tends to indicate a dermal influence in this disease. Any change in blood flow, gaseous tensions and nutrition to the epidermis could conceivably disturb the enzyme systems in both epithelial cells and melanocytes. It is well known that changes in temperature, pH, and substrate concentrations affect enzyme actions. It is possible that tyrosinase could be influenced by such factors.

Haxthausen's transplantation experiments in vitiligo (9) appear to support the existence of a neurotrophic factor in the pathogenesis of vitiligo. Other authors (10, 11) have not agreed with this. Recently, Lerner *et al.* (12) succeeded in demonstrating that melatonin, a lightening agent for frog melanocytes, is present in human peripheral nerves. Other evidence of increased nervous activity are reports of increased sweating (13) as well as vasoconstriction (14) in the hypopigmented areas. In addition, the association of emotional and traumatic shock preceding the development of vitiliginous lesions is not an unusual occurrence.

The hormonal influence on melanin pigmentation is well documented experimentally (15). Even though it is not certain whether the pituitary hormones and melatonin affect human pigment cells in the same way as they do in the aquatic animals, there is evidence to support the concept that hormones do influence human pigmentation.

One of our cases with rather extensive vitiligo exhibited an almost complete repigmentation within 3 months after a subtotal gastrectomy. This was a 36 year old white female who had extensive vitiligo of 22 years' duration. Under the influence of methoxsalen and ultraviolet light therapy she repigmented only moderately but not completely. The patient voluntarily discontinued her therapy and in a few months lost all the pigment she had gained during the therapy. She had a duodenal ulcer and atrophic gastritis with marked hypochlorhydria. There was no evidence of pernicious anemia. She was operated 4 months ago. The patient stated that she noticed spontaneous repigmentation soon following her operation. When she was seen approximately 3 weeks ago in our clinic, she had repigmented more extensively than she ever had during therapy with methoxsalen and ultraviolet light. The findings in this case are interesting when one thinks of the increased incidence of pernicious anemia in vitiligo reported by Allison and Curtis (16).

The existence of the hyperpigmented borders around vitiliginous areas and the presence of larger and more dendritic melanocytes in these hyperpigmented borders are findings which are worthy of note. It has been demonstrated that pigment cells enlarge and become more dendritic following various types of stimulation (17, 18); therefore, it is difficult to understand why the epithelial cells in the vitiliginous areas do not receive pigment from the very active melanocytes adjacent to them in the hyperpigmented border. We have demonstrated pigment in epithelial cells of normal skin which has been stimulated by ultraviolet light. One must postulate some transfer of pigment from the melanocytes to the epithelial cells. In vitiligo one could postulate some type of block between the dendrites of the melanocytes and the epithelial cells. Staricco (18) reported no increase in pigmentation in basal cells following stimulation by thorium X. This was interpreted by Staricco and Pinkus (2) to indicate a temporary block under pathologic conditions, which either does not permit the melanocytes to discharge their granules, or which, perhaps more probably, prevents the malpighian cells from accepting pigment.

The mechanism of pigment transfer, whether accomplished by simple phagocytosis or by a cytocrine process as proposed by Masson (4), has not been confirmed. Our time-lapse cinematographic observations in tissue culture preparations of human epidermal cells (19) tend, however, to support the latter theory.

It is not known whether repigmentation is initiated by the reactivation of "inactive" melanocytes in the vitiliginous skin or whether it is the result of the proliferation and migration of active melanocytes from the normal skin and the hair matrix melanocytes to the diseased areas. The repigmentation appears to result from the coalescence of perifollicular pigmentation and inward extension of the pigmented border at the periphery of the vitiliginous patch. We are inclined to favor the migration theory. In addition, the observation of return of pigmentation in vitiligo following dermabrasion (20) is more evidence in favor of the migration of the melanocytes from the hair matrix cells. The spread of pigment across the scar from a partially excised nevus is in all probability another example of melanocyte migration (Fig. 18).

Becker (21) studied the effect of combined psoralen and ultraviolet light on normal skin and concluded that the main effect of this therapy was to increase the thickness of the stratum corneum of the epidermis. It was his opinion that the increased pigmentation which resulted was due to the decreased shedding of the pigment from the surface of the epidermis. It is well known that a thickened epidermis offers good protection against sunburn while increased pigmentation or tanning which follows later serves as additional protection (22). At least part of the beneficial effect of psoralen on vitiligo could then be attributed to the protection against sunburn which permits the individual to tolerate longer exposure to light. By the same token melanocytes receiving longer exposure to sunlight would be more apt to form pigment. It is hard to believe that the entire systemic effect of psoralens can be explained by the mechanism just mentioned. It would indeed be interesting to compare the effect of oral and parenteral psoralens, should the latter become available for investigative studies.

A different mechanism is involved in the topical use of psoralens. Psoralens are well known photosensitizing agents. When the skin is painted with a solution of this drug and subsequently exposed to sunlight, a sunburn is produced more readily than in untreated skin. It is possible that the inflammatory reaction with associated hyperemia and increase in skin temperature results in the stimulation of the melanogenic activity in the melanocytes. This reaction may well be comparable to post-inflammatory hyperpigmentation which occurs in normal skin.

In the study of vitiligo one question seems to be of prime importance: On what factors does a favorable response to therapy depend? We feel that there are 3 main requisites: 1) melanocytes which are capable of forming pigment under stimulation must be present in the diseased sites, 2) there must be the capacity for the proliferation and/or migration of active melanocytes from adjacent normally pigmented areas to the vitiliginous areas, and 3) the ability of the epithelial cells to receive pigment from the melanocytes must be intact. Our results appear to indicate that all three factors are involved. The presence of silver positive granules in the epidermal cells with or without typical dopa-positive melanocytes in the diseased skin serves as indirect evidence for the existence of a potentially active tyrosinase system in the melanocytes of that area. The presence of these granules also appears to indicate the ability of the melanocytes to transfer pigment to the epithelial cells of the epidermis.

CONCLUSIONS

Data from cytological studies of 18 cases of vitiligo are presented. Included are 15 cases of typical vitiligo, 2 cases of congenital vitiligo and 1 case classified as pseudovitiligo.

Our results appear to indicate that a favorable response to combined methoxsalen and ultraviolet light therapy depends on 3 basic mechanisms: 1) the presence of melanocytes which are capable of forming pigment (shown by dopa reaction), 2) the capacity for proliferation and/or migration of active melanocytes from adjacent pigmented areas to vitiliginous areas, and 3) the ability of epithelial cells to receive pigment from the active melanocytes (shown by silver technic).

We would like to propose that a factor, equal in importance to the presence of melanocytes capable of forming melanin pigment, is operative in cases of vitiligo which fail to repigment following methoxsalen therapy. It is our opinion that

this factor is closely related to the transfer of pigment from the melanocytes to the epithelial cells. We believe that the best method for demonstrating this ability of pigment transfer is by silver staining.

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DISCUSSION

Dr. Samuel Becker (Los Angeles, Cal.): This very interesting presentation illustrates the advantages of the use of some of the modern methods in the study of the pigment cells.

Years ago I studied patches of vitiligo with the dopa reaction *en bloc*, and with Masson trichrome stain. I found after ultraviolet therapy there is strong dopa reaction at the border, that the melanocytes are branched with quite an inflammatory infiltrate extending up into the epidermis. In the center of the lesion, there was no inflammatory infiltrate. The melanocytes could be seen as very small cells in between the basal cell.

Dr. Hu does not mention the duration of the vitiliginous plaques. I think that is an important consideration. If the plaque has only been there from six months to a year it is not difficult to produce repigmentation. If it has been there for 20 years, it is practically impossible.

Dr. Leon Goldman (Cincinnati, Ohio): In the vein which Dr. Becker just mentioned, I would like to ask Dr. Hu about her concept of migration of melanocytes into a scar. I think that is of clinical importance. In conferences with the plastic surgeons about the complete excision of junctional nevi especially in the prepuberty period, and more so in infants, there have been a number of cases in which pigment has recurred in the exact center of the scar. This could not be due to incomplete excision or could not be due to any deep appendageal source, or anything like that at all. The question of the migration of melanocytes and transference into junction nevi was considered although we had no proof. I would like to ask Dr. Hu whether it does occur more easily in children and whether it could be responsible for the recurrence in the post operative scar.

Dr. George C. Andrews (New York, N.Y.): I hope that it will not be improper to inject a little clinical material in this discussion of Dr.

Hu's interesting paper. Recently in Buenos Aires, Professor Aaron Kaminsky at the Israel Hospital demonstrated some 20 cases of vitiligo that had been treated by tattooing with Solganal B (Schering) which is a gold preparation. They are convinced they get better results when they use Solganal B than from tattooing alone. The cases were all cured or practically cured of the lesions on the face and neck. But they get no benefits on the hands and wrists.

I have, since coming home, tattooed with Solganal B, cases on the face and neck with excellent results. Usually eight or ten weekly treatments brings back the pigment entirely. I mention this in connection with Dr. Hu's paper. I wonder if she has any explanation as to why you do not get clinical improvement on the hands and wrists.

DR. HERMANN PINKUS (Monroe, Mich): Dr. Staricco in our department has just concluded some work showing that the outer root sheath of hair follicles contains inactive melanocytes, and that these, and possibly melanocytes of the hair matrix move upward after planing operations and repopulate the epidermis. This will be published soon.

DR. FUNAN HU (in closing): I would like, first of all, to thank Drs. Becker, Goldman, Andrews and Pinkus for their comments and discussions.

With regard to Dr. Becker's question, in the two patients who repigmented rather extensively, one had the disease for 3 months prior to therapy, while the other had it for 30 years. It appears from these two examples that the duration of the disease makes little difference in the capacity to repigment. The patient who repigmented extensively following a subtotal gastrectomy also had the disease for more than 20 years.

I do feel that the melanocytes are capable of migration. The presence of pigmented spots in the scar following partial removal of a pigmented nevus, the repigmentation of vitiliginous patches as the result of coalescence of perifollicular pigmentation and inward extension of the pigmented border, and the return of pigment in vitiligo following dermabrasion all appear to indicate melanocyte migration.

I cannot answer Dr. Goldman's question as to whether or not migration of melanocytes occurs more easily in children, since my experience in dealing with removal of nevi in children is rather limited. However I am inclined to believe that the recurrence of pigment in the postoperative scar could easily be due to the migration of pigment cells from the neighboring parts.

I am glad that Dr. Andrews asked the question about the failure of improvement of vitiliginous patches of hands and wrists. The presence of silver positive cells in the vitiliginous patch only indicates the likelihood of repigmentation in that particular area. It does not mean that all the lesions in the patient will improve similarly. We all have seen patients who may repigment readily in some areas while other areas fail to repigment no matter what you do.

From the observations on the few cases included in the present study it is not possible to draw any definite conclusions other than a few postulations as made in this presentation. It is hoped that our findings may be substantiated by further study and follow up of a large series of cases so that eventually definite conclusions can be made.